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Pseudonannolene canastra sp. nov. (Diplopoda, Spirostreptida) – a new troglobitic millipede from the southwestern state of Minas Gerais, Brazil

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Abstract

Pseudonannolene is a neotropical genus of millipedes distributed in Brazil, Argentina, Paraguay, Uruguay and Bolivia. Generally, species of *Pseudonannolene* are considered troglophilic, *i.e.*, they can establish a source population in both subterranean and superficial habitats. Among the 60 species known, 49 are found in Brazil; out of these, 25 occur in caves but only three are considered troglobitic (source population exclusively subterranean). This study aims to describe the fourth troglobitic species of *Pseudonannolene* from Brazil, and the first one from the region of Serra da Canastra, in the southeastern part of Brazil.

Keywords

diplopod, hypogean, neotropical, troglomorphism, Serra da Canastra

Introduction

Pseudonannolene Silvestri, 1895 (Spirostreptida, Pseudonannolenidae) is a neotropical genus of millipedes distributed in Brazil, Argentina, Paraguay, Uruguay and Bolivia (Schubart 1944; Mauriès 1987; Enghoff et al. 2015). The diagnostic feature of this

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genus is the presence of a longitudinal division of the prementum of the gnathochilarium (Enghoff et al. 2015). Generally, species are considered troglophilic (Trajano et al. 2000; Enghoff et al. 2015), *i.e.*, they can establish a source population in both hypogean (subterranean) and epigean (superficial) environments with individuals that can move between these habitats, thus promoting the flow of selected genes in both environments (Trajano 2012; Trajano and Carvalho 2017).

Among the 60 species known, 49 were reported in Brazil and 25 among these occur in caves, while three are considered troglobitic (Iniesta and Ferreira 2014) *i.e.*, the source population is exclusively subterranean (Trajano and Carvalho 2017). Most of the described species are in the states of Minas Gerais and São Paulo (Gallo and Bichuette 2019). In Brazil, the known troglobitic species include *P. spelaea* Iniesta & Ferreira, 2013, from Pará state, *P. ambuatinga* Iniesta & Ferreira, 2014, and *P. lundi* Iniesta & Ferreira, 2015; these last recorded in the Minas Gerais state. For these species, the troglomorphisms, *i.e.*, modifications in morphological characteristics associated with subterranean isolation (Christiansen 1962), include reduction in body pigmentation, variation in number of ommatidia, body size, and antennae elongation (Iniesta and Ferreira 2015).

Despite little knowledge about this group, two cave species, *P. ambuatinga* and *P. spelaea*, are included in the List of Brazilian Threatened Fauna under Critically Endangered (CR) and Endangered (EN) categories, respectively (Chagas Jr et al. 2018a, b). This classification is based on the IUCN (International Union for Conservation of Nature) rules and adapted to the Brazilian criteria.

This study aims to describe the fourth troglobitic species of *Pseudonannolene* from Brazil and the first one from the region of Serra da Canastra in southeast Brazil and discuss its troglomorphisms and conservation status.

Materials and methods

Study area

The Gruta do Tesouro cave (Figure 1) is located in the municipality of São Roque de Minas, in the region of Serra da Canastra, Minas Gerais state, 350 km away from the capital Belo Horizonte. The climate of the region, according to Köppen's classification, is Cwa with dry winters and warm summers (Alvares et al. 2013), with rainy season occurring from October to April and dry season from May to September. The vegetation is composed of Cerrado (savanna-like) with riparian and mesophilic forests (Batista et al. 2018).

The Gruta do Tesouro cave is composed of limestone rock belonging to the Bambuí geomorphological unit and has 1,320 m of passageways (Figure 2A). The cave is outside the Serra da Canastra National Park (PNSC) and its surroundings are affected by the pastures adjoining the cave entrance (Figure 2B); uncontrolled tourism is another factor impacting the cave-dwellers. The specimens occur on the riverbank substrate in an aphotic zone, *i.e.*, in complete darkness (Figure 2C) with bat guano piles close by.

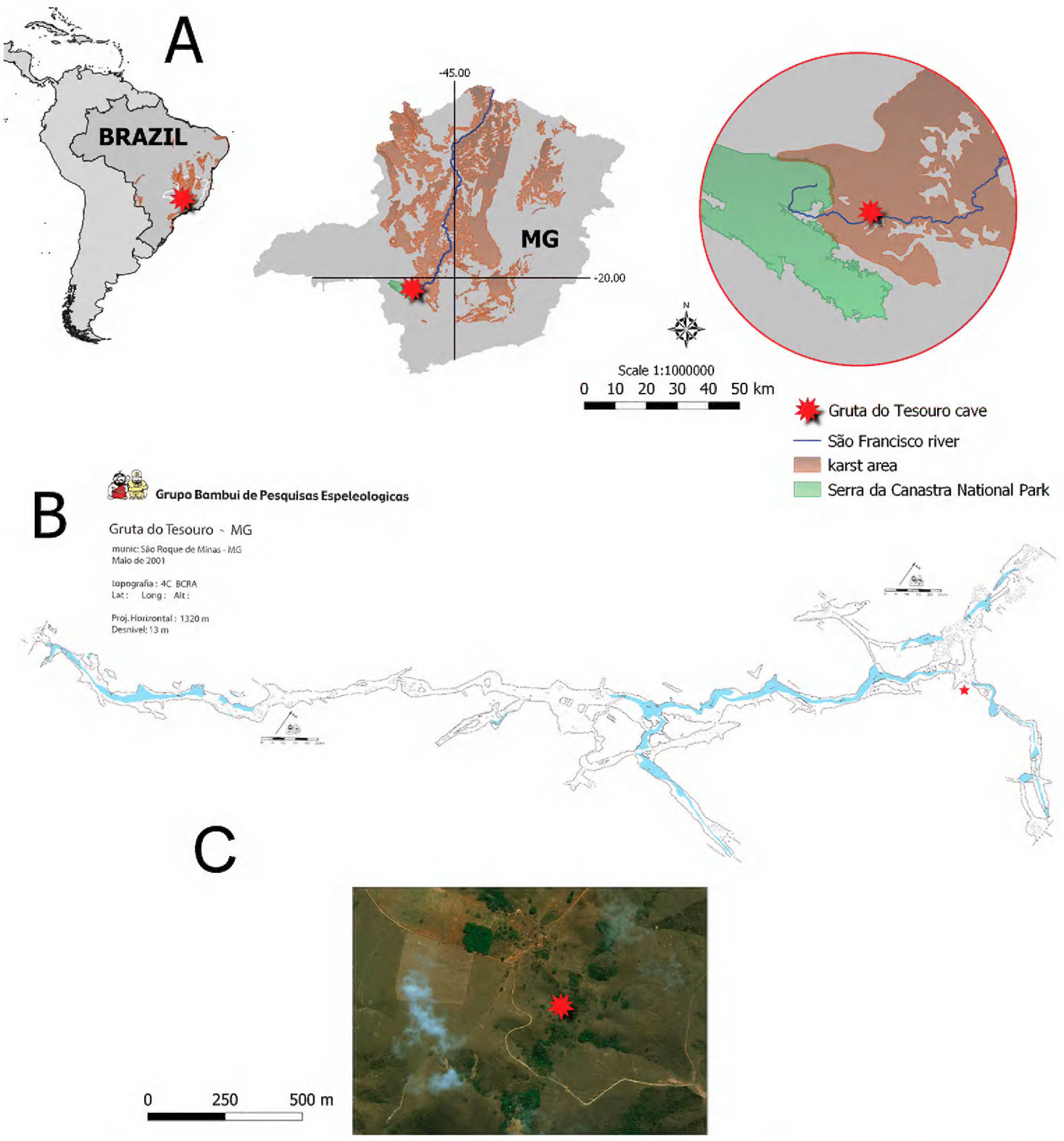


Figure 1. Study area **A** Gruta do Tesouro geographical location (DM von Schimonsky) **B** cave map; the star corresponds to the entrance used to access the cave in this study (Grupo Bambuí de Pesquisas Espeleológicas) **C** satellite image; the star shows the entrance of the cave (Bing).

Collections

The specimens were manually captured by active search and placed in a non-toxic plastic container and taken to the Laboratório de Estudos Subterrâneos at Universidade Federal de São Carlos (LES/UFSCar). The specimens were observed daily for seven days and photographed alive at laboratory. The specimens were then fixed in 70 % ethanol and photographed. The images were captured on a Leica DFC295 camera coupled to a Leica M205C stereomicroscope with Planapo 1.0× lens and produced by mounting multiple images using the LAS software (Leica Application Suite) V3.7. The holotype was analyzed using a Scanning Electron Microscope (FEI Quanta 250, FEG-SEM) in

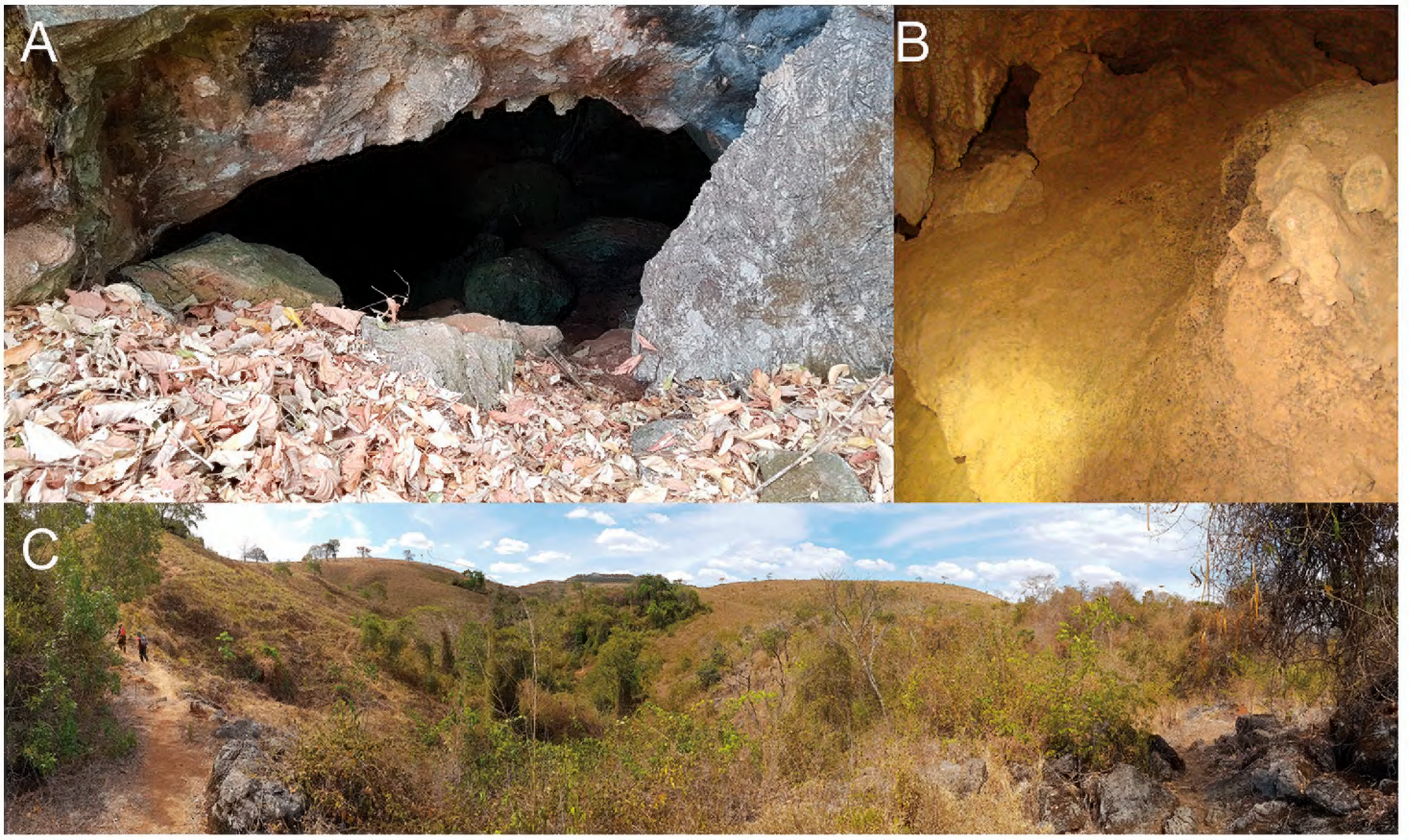


Figure 2. Study Area **A** principal cave entrance **B** riverbank substrate where the specimens were found **C** landscape view of Gruta do Tesouro cave surroundings. (Photos a and c DM von Schimonsky).

Table 1. Species deposited at the Museu de Zoologia da Universidade de São Paulo (MZUSP).

Species	Collection number
P. imbirensis Fontanetti, 1996	MZUSP1094
P. tocaiensis Fontanetti, 1996	MZUSP1095
P. halophila Schubart, 1949	MZUSP1096
P. maritima Schubart, 1949	MZUSP1098
P. leucomelas Schubart, 1947	MZUSP1099
P. tricolor Brölemann, 1902	MZUSP1100
P. chaimowiczi Fontanetti, 1996	MZUSP1101
P. leucocephalus Schubart, 1944	MZUSP1102
P. ophiiulus Schubart, 1944	MZUSP1103
P. silvestris Schubart, 1944	MZUSP1105
P. urbica Schubart, 1945	MZUSP1106
P. meridionalis Silvestri, 1902	MZUSP1115
P. albiventris Schubart, 1952	MZUSP1116
P. sebastianus Brölemann, 1902	MZUSP1118
P. paulista Brölemann, 1902	MZUSP1121

a low-vacuum mode. All individuals that did not present pairs of legs in all segments of the body, that is the growth zone, were considered juveniles (Makarov 2015). The gonopods and the first leg-pair of the male were drawn with the program Adobe Illustrator CC 2014 and the map prepared in the QGIS program version 3.4. The studied material was deposited in the zoological collection at the LES / UFSCar. We examined the species deposited at the Museu de Zoologia da Universidade de São Paulo (**MZUSP**) (listed in Table 1); besides, the original descriptions of all species of *Pseudonannolene* (eg. Schubart 1944, 1945, 1947, 1949, 1958, 1960; Silvestri 1895, 1897,1902; Bröle-

man 1902, 1929; Mauriès 1974, 1987; Fontanetti 1996a, b, 2000; Iniesta and Ferreira 2013a, b, c, 2014, 2015) were used. Thus, we use the terminology used by Schubart (1944, 1945, 1947, 1949, 1958, 1960) and Fontanetti (1996a, b) to refer to the components of the diagnostic structures (gonopod and first leg-pair of male).

Results

Taxonomy
Order Spirostreptida Brandt, 1833
Family Pseudonannolenidae Silvestri, 1895
Genus *Pseudonannolene* Silvestri, 1895

***Pseudonannolene canastra* Gallo & Bichuette, sp. nov.**
http://zoobank.org/D1CDA013-785D-4800-A746-87BF93FE48C8
Figs 3–6

Materials Examined. ***Holotype*:** Brazil • ♂; Gruta do Tesouro cave, São Roque de Minas, Minas Gerais, Brazil; 26.IX.2017; Fernandes CS, Gallo JS, von Schimonsky DM leg.; LES15282. ***Paratypes*:** Brazil • 3♀ (two adults and one juvenile); same locality; 15.VIII.2014; by Bolfarini MP and Zepon T leg.; LES15283 • 1♂ 2♀ (juveniles); same locality, data and collectors as for holotype; LES15284.

Etymology. The name "canastra" is in allusion to a kind of an ancient chest. This name is also used to refer to the region where the Gruta do Tesouro cave is locate, Serra da Canastra, that is characterized by a chain of mountais shaped like canastra. Here, we use Canastra as a noun in apposition.

Diagnosis. *Pseudonannolene canastra* sp. nov. has wide coxa of gonopods resembling those of *P. microzoporus* Mauriès, 1987, while its solenomerite shape is similar to that of *P. maritima* Schubart, 1948. However, *P. canastra* sp. nov. differs from these species in characteristics such as the round shape of the gonopod coxa, the telopodite with a wide base, reduced dentiform processes, different number of spines in the inner part of the coxa (three in the left coxa and four in the right coxa in caudal view), and a divergent pre-femoral process with reduced and flaky pre-femur prolongations. Externally, these species are quite distinct. *Pseudonannolene microzoporus*, considered troglophilic, has a uniform light brown pigmentation (Mauriès 1987) while *P. maritima*, recorded only in epigean environment, is brownish or brownish-black, and the head and the first two segments are always lighter, with nearly yellowish color (Schubart 1948).

Holotype description (male). Body length 36.26 mm, ring diameter 1.98 mm, with 59 rings (counting the collum and the telson) (Table 2) without anterior or posterior tapering of the body; the prozonite is shorter (0.04 mm) than the metazonite (0.33 mm); metazonite has thin striae in the ventrolateral portion of all the rings; the gnathochilarium is typical of the genus *Pseudonannolene* with longitudinally divided promentum (Enghoff et al. 2015); head has a posterior suture from the collum to the line of the eyes.

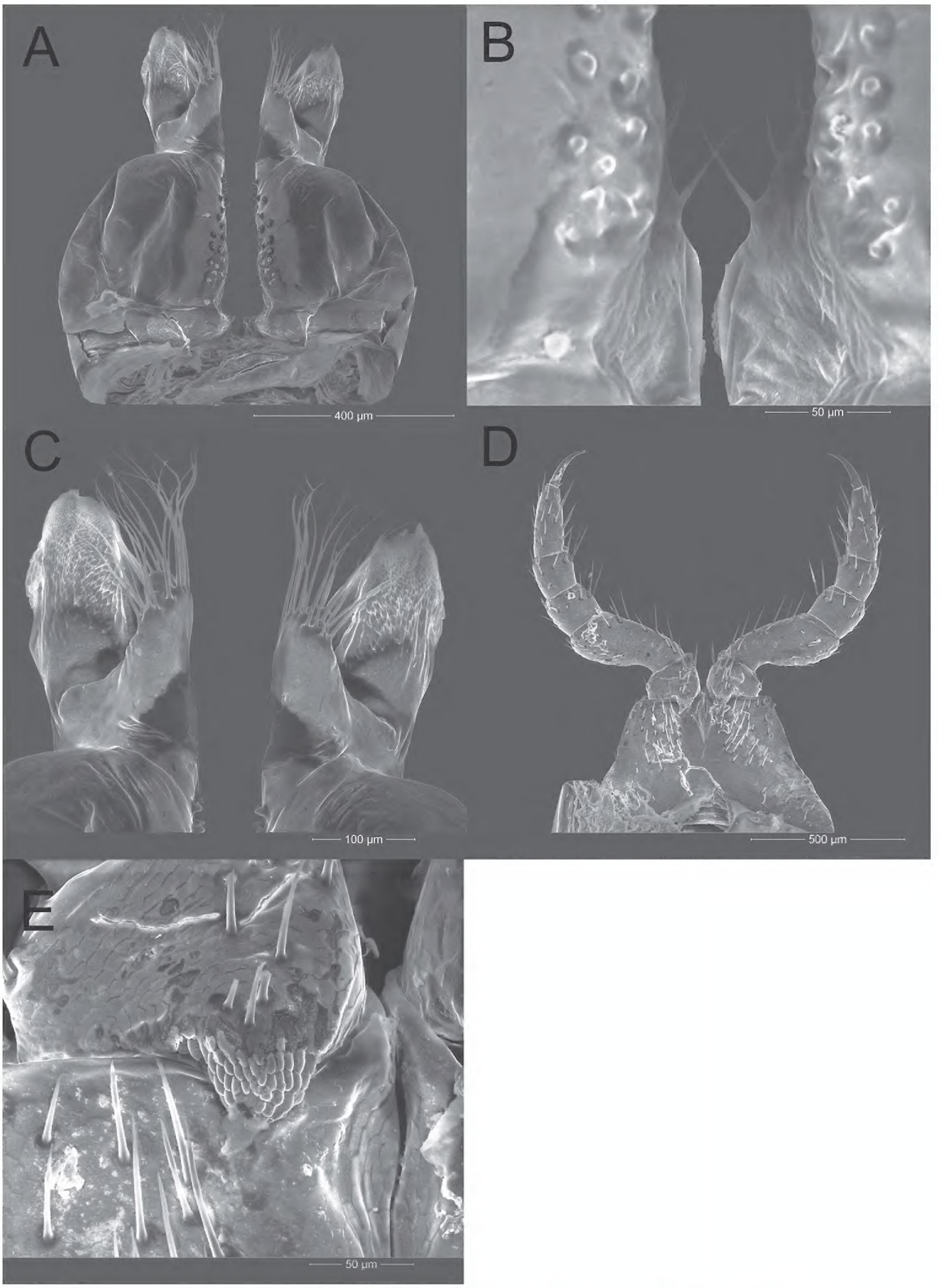


Figure 3. Gonopod an Fist pair of holotype legs by Scannig Electron Microscope (SEM) **A** caudal view of gonopod **B** detail of the internal spines and dentiform process **C** detail of solenomerite e telopodite **D** caudal view of male first pair of legs **E** detail of pre-femural process. Photo: LBR Fernandes.

Table 2. Morphological data of all the specimens analyzed for description. H: holotype, P: paratype, Ad: Adult, Jv: juvenile, F: female, M: male, R: right, L: left, O: oval, T: triangular, NO: could not be observed.

***Pseudonannolene canastra* sp. nov.**	**H**	**P001**	**P002**	**P003**	**P004**	**P005**	**P006**	**P007**
Age	Ad	Ad	Ad	Jv	Jv	Jv	Jv	Ad
Sex	M	F	F	F	F	F	M	F
Length (mm)	36.23	44.82	54.49	30.47	17.24	20.13	34.66	46.20
N° of rings	59	58	67	NO	43	46	56	60
N° of ommatidia	R:22	R:20	R:24	R:22	R:8	R:11	R:19	R:18
	L:24	L:20	L:28	L:14	L:8	L:11	L:16	L:22
Shape	O	O	O	O	O/T	O/T	O	O
Anal plate setae	2+2	NO	NO	NO	2+2	2+2	2+2	2+2

***Pigmentation*:** In life, the pigmentation of the head, antenna, legs, and body rings is light yellow with no difference in the coloration of the prozonite and metazonite; the ommatidia and ozopores are reddish (Figure 4). Pigmentation in 70 % ethanol: The head, rings, and legs darken after fixation (brown coloration), the eyes become brown like the head by losing the reddish coloration. Only the antenna maintained the original color (Figure 5).

***Antenna*:** Slender (2.3 mm length), covered by fine white setae almost transparent, with some setae larger than others; antennomeres longer than width; the third antennomere is the largest; the sixth antennomere is the widest with a round shape and four sensory cones in the end. The antenna length ratio to the head 1.7 (from the beginning of the suture from labrum) while diameter ratio 1.17.

***Eyes*:** oval-shaped lateral eyes area 0.09 mm^2, lengths 0.44 mm and 0.43 mm (right and left, respectively); width 0.3 mm; twenty-two ommatidia (Richter et al. 2010) on the right side and 24 ommatidia on the left side (Table 2).

Gonopodium (Figure 6A–D): The coxa is round, 0.45 mm long and 0.34 mm wide; the dentiform processes reduced in number and size (Figure 3A) with three internal spines (or processes, Iniesta and Ferreira 2013) on the left side and four on the right side (Figure 3B, one of the spines is not visible in the figure); the solenomerite end rounded and scaly (Figure 3C) (0.24 mm long, 0.12 mm wide), corresponding to 0.54 and 0.35 of the coxa length and width, respectively; the telopodite smaller than the solenomerite in length (0.122 mm) but larger in width (0.139 mm) and corresponds to 0.51 and 1.17 of the solenomerite length and width respectively; it measures 0.15 mm in length and has a wide base (with thin setae) that narrows gradually (Figure 3D).

First pair of legs (Figure 6 E–F): coxa longer than wide (0.58 mm length, 0.28 mm width); setae are concentrated in the distal region of the coxa and few setae are found in the proximal region (Figure 6 D); the coxa base is slightly narrow; the pre-femur wider (0.18 mm) than length (0.15 mm); strong setae concentrate on the inner side of the pre-femur; pre-femoral process divergent, as small, rounded and scaly extensions in the base of the pre-femur (Figure 3E); femur (0.27 mm), post-femur

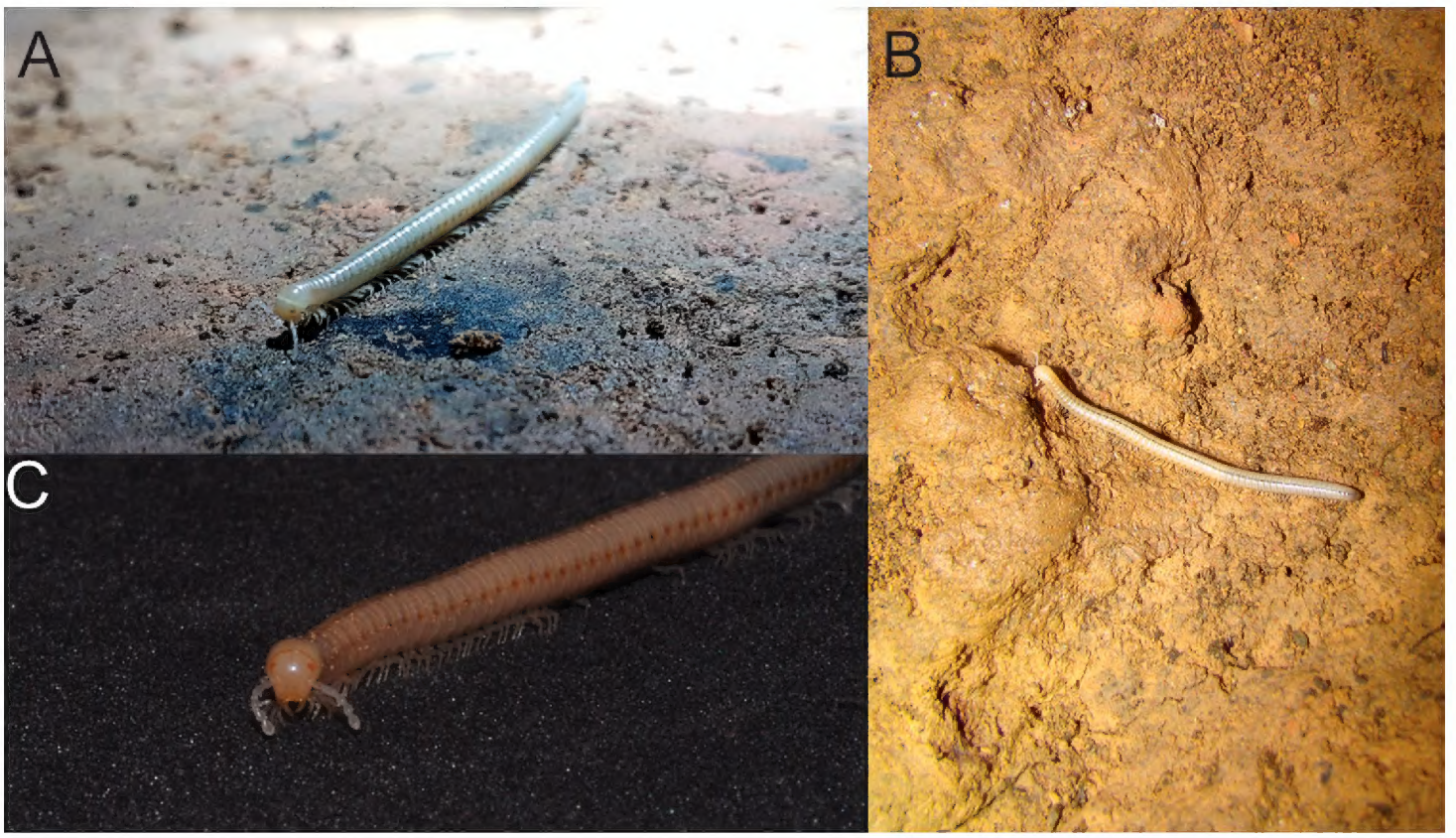
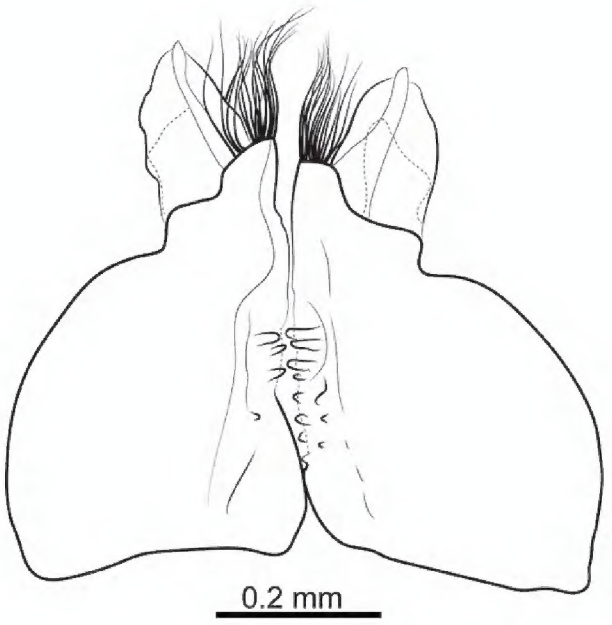


Figure 4. *Pseudonannolene canastra* sp. nov. live specimens **A, B** specimens in natural environment **C** at laboratory, specimen darkened after contact with artificial light. Photos: **A** DM von Schimonsky and **C** DJ Tomaz.

Figure 5. *Pseudonannolene canastra* sp. nov. in left lateral view, preserved in ethanol 70%. Photo: LBR Fernandes.



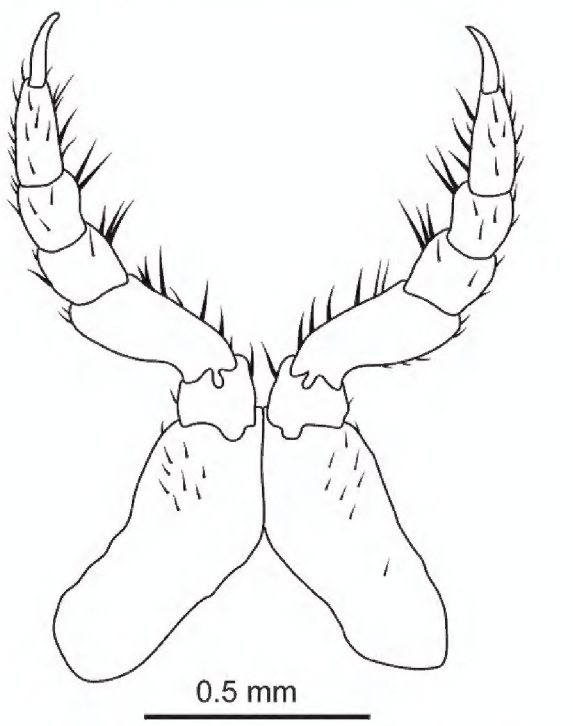


Figure 6. Gonopods and first pair of legs of *Pseudonannolene canastra* sp. nov. **A, B** scheme and stereomicroscopic photograph of the gonopods in caudal view **C, D** scheme and photograph in stereomicroscope of the gonopods in oral view **E, F** schematic and stereomicroscopic photograph of the first pair of legs in oral view. Photo: LBR Fernandes.

(0.15 mm), tibia (0.13 mm), and tarsus (0.22 mm) with thick, elongated setae; the tarsal claw is 0.15 mm long.

***Walking legs* (*mean values*):** total length 1.79 mm; coxa (0.21 mm), pre-femur (0.26 mm), femur (0.33 mm), post-femur (0.27 mm); tibia (0.23 mm); tarsus (0.34 mm) with setae on the inner side; all podomeres longer than wide; the tarsal claw 0.14 mm length.

***Telson*:** The anal valve has 2 + 2 setae, no sulcus; the pre-anal sclerite does not extend beyond the anal valve; the subanal plate lacks projection (Table 2).

Distribution. An endemic species of Gruta do Tesouro cave, São Roque de Minas, Minas Gerais, Brazil.

Observations. The juveniles, *i.e.* individuals that did not present pairs of legs in all segments of the body (Makarov 2015), show symmetry in the number of ommatidia; eyes are more triangular than oval. The other characteristics of the body are the same as those of the adults. For this species, the females are larger than the males.

Discussion

Pseudonannolene canastra sp. nov. is a Spirostreptidan troglobitic millipede restricted to the Gruta do Tesouro cave, with non-occurrence on epigean (surface) habitats, reduction in pigmentation and displaying a classical troglomorphism *i.e.*, the convergence in morphological traits related to isolation in the subterranean environment (Christiansen 1962). Millipedes are a conservative group (Causey 1960) and "pre-adapted" (*sensu* Arnold 1994) to subterranean habitats. Thus, the inference of morphological modifications associated with the isolation in subterranean environment for the Brazilian *Pseudonannolene* millipedes is observed to a lesser extent. Only nine troglobitic (*i.e.* exclusive to the subterranean realm) millipedes have been identified from Brazil: five of them belonging to the order Polydesmida (*Dobrodesmus mirabilis* Shear, Ferreira & Iniesta, 2016; *Leodesmus yporangae* (Schubart, 1946); *Phaneromerium cavernicolum* Golovatch & Wytwer, 2004; *Peridontosmella alba* Schubart, 1957; and *Yporangiella stygius* Schubart, 1946), three from the order Spirostreptida (*P. ambuatinga*, *P. lundi* and *P. spelaea*), and one from the order Glomeridesmida (*Glomeridesmus spelaeus* Iniesta & Wesener, 2012).

Adult millipedes generally have dark pigmentation, while the juveniles can be lighter because of the ecdysis process (Makarov 2015). However, *P. canastra* sp. nov. maintains light pigmentation in adult stage. The reduction of integument pigmentation was observed in the troglobitic species *P. ambuatinga*, *P. lundi*, and *P. spelaea*. Darkening of the pigmentation of *P. canastra* sp. nov. was also observed when they were exposed to artificial lighting. This darkening has already been reported for *P. spelaea* (Iniesta and Ferreira 2013).

Some cave-dwelling species of the genus *Plusioglyphiulus* (Spirostreptida, Cambalopsidae) present ommatidia varying from four to 13; this was not considered as a troglomorphic trait (Golovatch et al. 2009). Some examples of troglobitic species belonging

to the order Spirostreptida are *Cambala speobia* Chamberlin, 1953; *C. reddelli inornatus* Causey, 1964; and *Mexicambala russelli* Causey, 1964; they have been identified from caves in North America. These species show troglomorphic traits such as lack of eyes (*C. speobia* and *M. russelli*) or reduced number of ommatidia ca. 2–7 (*C. reddelli inornatus*). The species of the Pseudonannolenidae family have four to 45 ommatidia; they are rarely absent (Enghoff et al. 2015). Following this pattern, we do not consider that *P. canastra* sp. nov. has a reduced number of ommatidia. Besides, ommatidia are absent in the pseudonanolenid *Typhlonannolene adaptus* Chamberlin, 1923, an epigean species, which reinforces the idea that the reduction (until its complete absence) in the number of ommatidia is not simply related to the isolation in the subterranean environment. Therefore, it is difficult to infer if this character-state truly represents a troglomorphism.

There are some gaps in the description of the *Pseudonannolene* species. One of them is related to the antenna measurements. Forty-one percent of descriptions do not mention the exact measurement or only report that the antenna is long. For the remaining 59% of the described species, the ratio between the antenna length and the body diameter ranges from 0.59 for *P. maritima* to 1.55 for *P. tricolor* – the last species is considered troglophilic. Considering the troglobitic species – *P. spelaea*, *P. ambuatinga* and *P. lundi*, the ratio ranges from 0.86 to 1.04 (*P. ambuatinga*), 1.03 to 1.08 (*P. lundi*), and 0.93 to 1.06 (*P. spelaea*). The antenna length of 2.3 mm in *P. canastra* sp. nov. is 1.17 of its body diameters, the largest for the troglobitic species. However, this ratio is also large in the troglophilic species *P. microzoporus* (1.27), *P. strinatii* (1.23 to 1.5) and *P. tricolor* (1.52 to 1.55), for example, even larger than that observed in the troglobitic species.

Considering the body dimensions, the order Spirostreptida ranges from 0.6 to 20 mm in body diameter (Enghoff et al. 2015). The troglobitic species *Cambala reddelli inornatus*, *M. russelli* and *C. speobia* have a body diameter of 1.0, 1.3, and 1.9 mm, respectively. For the Pseudonannolenidae family, the body is considered slender compared to the other spirostreptidians, with diameters varying from 0.7 to 6 mm (Enghoff et al. 2015); most of the *Pseudonannolene* species have a diameter ranging from 2 to 5 mm. The adult *P. canastra* sp. nov. shows a body diameter of 1.97 mm and is thinner when compared with the other two troglobitic species *P. ambuatinga* (2.4 to 3.36 mm), and *P. lundi* (2.4 to 2.8 mm). However, it is slightly larger than the troglobitic *P. spelaea* (1.74 to 1.88 mm) in body diameter. Some troglophilic species like *P. leucocephalus* and *P. taboa* show their body diameter ranging from 1.4 to 2.2 mm and 5.5 to 6.1 mm respectively. So, we cannot infer whether the body diameter observed in *P. canastra* sp. nov. is a reduction and consequently a troglomorphism or not.

Endemism and conservation

The Serra da Canastra is known for its scenic beauty with large rocky walls, waterfalls, and its biological richness of fauna and flora (ICMBio 2018). However, the Serra da Canastra National Park (PNSC) is the only protected part of this region. The Gruta

do Tesouro cave is outside the perimeter of the PNSC and located on private property, therefore, it is unprotected. The farm owners manage tourist activities, and no management plan is implemented.

Pseudonannolene canastra sp. nov. is the first troglobitic species described for Serra da Canastra. The present species is geologically isolated from the other species described for the genus *Pseudonannolene* due to their occurrence inside the caves. The Municipality of São Roque de Minas is in the geomorphological unit of Bambuí, specifically in the formation of Paraopebas (Karmann and Sanchéz 1979). The nearest described species are from the Pains region located about 85 km from São Roque de Minas, inserted in the Sete Lagoas formation (another formation of the Bambuí geomorphological unit) (CODEMIG 2003).

Pseudonannolene canastra sp. nov. is mostly found in a specific substrate (the bank of a river) with high humidity; any change in its habitat is dangerous. This species is endemic to the Serra da Canastra region and restricted to the Gruta do Tesouro cave, which is threatened by uncontrolled tourism and deforestation for pasture. Samplings in other caves in the region were made and this species were not found. This can be justified by the limited dispersal power of millipedes (Hopkin and Read 1992; Golovatch and Kime 2009) resulting in a high degree of speciation, and the evolution of many endemic species (Hopkin and Read 1992). The troglobitic species described were found in very moist microhabitats in the caves, associated with drainage or speleothems and, bat guano and vegetable debris as food resources (e.g. Iniesta and Ferreira 2015). Because of these conditions, we carried out a preliminary evaluation concerning the status of conservation of this species following the IUCN (International Union of Conservation of Nature) classification. *Pseudonannolene canastra* sp. nov. was classified as Critically Endangered (CR) with the criteria B1ab (iii): **B** being the restricted geographical area; **B1** the occurrence extension of the taxon (<100 km^2 according IUCN, the present species has 51.9 km^2); **a** represents one location and **b(iii)** represents the continuous decline of habitat quality. In addition to *P. canastra* sp. nov., two more subterranean species are known from the Serra da Canastra (different taxonomic group) that has been sampled by LES team (Laboratório de Estudos Subterrâneos) since 2009 (Gallão and Bichuette 2018). This shows the potential and biospeleological importance of the region, as well as the need for adequate protection and management of the cave, its surroundings, the watershed and landscape (Gallão and Bichuette 2018).

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